

SEQUENCE LISTING

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 Ho, Mitchell
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 The Government of the United States
 as represented by The Secretary of the
 Department of Health and Human Services

<120> Mutated Anti-CD22 Antibodies and Immunoconjugates

<130> 015280-500100US

<140> US 10/580,635
 <141> 2006-05-25

<150> US 60/525,371
 <151> 2003-11-25

<150> WO PCT/US04/39617
 <151> 2004-11-24

<160> 30

<170> PatentIn Ver. 2.1

<210> 1
 <211> 321
 <212> DNA
 <213> Mus sp.

<220>
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal
 antibody light chain variable region (VL)

<220>
 <221> CDS
 <222> (1)..(321)
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal
 antibody light chain variable region (VL)

<400> 1
 gat atc cag atg acc cag act aca tcc tcc ctg tct gcc tct ctg gga 48
 Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15

gac aga gtc acc att agt tgc agg gca agt cag gac att agc aat tat 96
 Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
 20 25 30

tta aac tgg tat cag cag aaa cca gat gga act gtt aaa ctc ctg atc 144
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45

tac tac aca tca ata tta cac tca gga gtc cca tca agg ttc agt ggc 192
 Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

agt ggg tct gga aca gat tat tct ctc acc att agc aac ctg gag caa 240
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
 65 70 75 80

gaa gat ttt gcc act tac ttt tgc caa cag ggt aat acg ctt ccg tgg 288
 Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
 85 90 95

acg ttc ggt gga ggc acc aag ctg gaa atc aaa 321
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 2
 <211> 107
 <212> PRT
 <213> Mus sp.

<220>
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal
 antibody light chain variable region (VL)

<400> 2
 Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15
 Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45
 Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
 65 70 75 80
 Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 3
 <211> 369
 <212> DNA
 <213> Mus sp.

<220>
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal
 antibody heavy chain variable region (VH)

<220>
 <221> CDS
 <222> (1)..(369)
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal
 antibody heavy chain variable region (VH)

<400> 3
 gaa gtg cag ctg gtg gag tct ggg gga ggc tta gtg aag cct gga ggg 48
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc gct ttc agt atc tat	96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr	
20 25 30	
gac atg tct tgg gtt cgc cag act ccg gag aag agg ctg gag tgg gtc	144
Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val	
35 40 45	
gca tac att agt agt ggt ggt ggt acc acc tac tat cca gac act gtg	192
Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val	
50 55 60	
aag ggc cga ttc acc atc tcc aga gac aat gcc aag aac acc ctg tac	240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr	
65 70 75 80	
ctg caa atg agc agt ctg aag tct gag gac aca gcc atg tat tac tgt	288
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys	
85 90 95	
gca aga cat agt ggc tac ggt agt agc tac ggg gtt ttg ttt gct tac	336
Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr	
100 105 110	
tgg ggc caa ggg act ctg gtc act gtc tct gca	369
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala	
115 120	

<210> 4
 <211> 123
 <212> PRT
 <213> Mus sp.

<220>
 <223> RFB4 mouse IgG1 anti-human CD22 monoclonal
 antibody heavy chain variable region (VH)

<400> 4
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr
 20 25 30
 Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
 35 40 45
 Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 5
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:carboxyl
terminal fragment binding KDEL recycling receptor
for transport of construct into cytosol from
endoplasmic reticulum

<400> 5
Lys Asp Glu Leu
1

<210> 6
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:carboxyl
terminal fragment binding KDEL recycling receptor
for transport of construct into cytosol from
endoplasmic reticulum

<400> 6
Arg Glu Asp Leu
1

<210> 7
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:RFB4 variable
light chain (VL) complementarity determining
region 1 (CDR1)

<400> 7
Gln Asp Ile His Gly Tyr
1 5

<210> 8
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:RFB4 variable
 light chain (VL) complementarity determining
 region 1 (CDR1)

<400> 8
 Gln Asp Ile Gly Arg Tyr
 1 5

<210> 9
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:RFB4 variable
 light chain (VL) complementarity determining
 region 1 (CDR1)

<400> 9
 Gln Asp Ile Arg Gly Tyr
 1 5

<210> 10
 <211> 6
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:RFB4 variable
 light chain (VL) complementarity determining
 region 1 (CDR1)

<400> 10
 Gln Asp Ile Ala Arg Tyr
 1 5

<210> 11
 <211> 3
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:RFB4 variable
 light chain (VL) complementarity determining
 region 2 (CDR2)

<400> 11
 Tyr Thr Ser
 1

<210> 12
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:RFB4 variable
light chain (VL) complementarity determining
region 3 (CDR3)

<400> 12
Gln Gln Gly Asn Thr Leu Pro Trp Thr
1 5

<210> 13
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 1 (CDR1)

<400> 13
Gly Phe Ala Phe Ser Ile Tyr Asp
1 5

<210> 14
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 2 (CDR2)

<400> 14
Ile Ser Ser Gly Gly Gly Thr Thr
1 5

<210> 15
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 2 (CDR2)

<400> 15
Ala Phe His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
1 5 10 15

<210> 16
<211> 16
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 3 (CDR3)

<400> 16

Ala Phe His Ser Gly Tyr Gly Thr His Trp Gly Val Leu Phe Ala Tyr
1 5 10 15

<210> 17

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 3 (CDR3)

<400> 17

Ala Phe His Ser Gly Tyr Gly Tyr Asn Trp Gly Val Leu Phe Ala Tyr
1 5 10 15

<210> 18

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 3 (CDR3)

<400> 18

Ala Phe His Ser Gly Tyr Gly Thr Thr Trp Gly Val Leu Phe Ala Tyr
1 5 10 15

<210> 19

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:RFB4 variable
heavy chain (VH) complementarity determining
region 3 (CDR3)

<400> 19

Ala Phe His Ser Gly Tyr Gly Ser Thr Tyr Gly Val Leu Phe Ala Tyr
1 5 10 15

<210> 20

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:mutated RFB4 VL
chain

<400> 20

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile His Gly Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45
Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65 70 75 80
Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
85 90 95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 21

<211> 123

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:mutated RFB4 VH
chain

<400> 21

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr
20 25 30
Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45
Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95
Ala Arg His Ser Gly Tyr Gly Thr His Trp Gly Val Leu Phe Ala Tyr
100 105 110
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 22
 <211> 345
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Pseudomonas
 exotoxin A cytotoxic fragment PE38 translocating
 and ADP ribosylating domains

<400> 22

Gly	Gly	Ser	Leu	Ala	Ala	Leu	Thr	Ala	His	Gln	Ala	Cys	His	Leu	Pro	1	5	10	15
Leu	Glu	Thr	Phe	Thr	Arg	His	Arg	Gln	Pro	Arg	Gly	Trp	Glu	Gln	Leu	20	25	30	
Glu	Gln	Cys	Gly	Tyr	Pro	Val	Gln	Arg	Leu	Val	Ala	Leu	Tyr	Leu	Ala	35	40	45	
Ala	Arg	Leu	Ser	Trp	Asn	Gln	Val	Asp	Gln	Val	Ile	Arg	Asn	Ala	Leu	50	55	60	
Ala	Ser	Pro	Gly	Ser	Gly	Gly	Asp	Leu	Gly	Glu	Ala	Ile	Arg	Glu	Gln	65	70	75	80
Pro	Glu	Gln	Ala	Arg	Leu	Ala	Leu	Thr	Leu	Ala	Ala	Ala	Glu	Ser	Glu	85	90	95	
Arg	Phe	Val	Arg	Gln	Gly	Thr	Gly	Asn	Asp	Glu	Ala	Gly	Ala	Ala	Asn	100	105	110	
Gly	Pro	Ala	Asp	Ser	Gly	Asp	Ala	Leu	Leu	Glu	Arg	Asn	Tyr	Pro	Thr	115	120	125	
Gly	Ala	Glu	Phe	Leu	Gly	Asp	Gly	Gly	Asp	Val	Ser	Phe	Ser	Thr	Arg	130	135	140	
Gly	Thr	Gln	Asn	Trp	Thr	Val	Glu	Arg	Leu	Leu	Gln	Ala	His	Arg	Gln	145	150	155	160
Leu	Glu	Glu	Arg	Gly	Tyr	Val	Phe	Val	Gly	Tyr	His	Gly	Thr	Phe	Leu	165	170	175	
Glu	Ala	Ala	Gln	Ser	Ile	Val	Phe	Gly	Gly	Val	Arg	Ala	Arg	Ser	Gln	180	185	190	
Asp	Leu	Asp	Ala	Ile	Trp	Arg	Gly	Phe	Tyr	Ile	Ala	Gly	Asp	Pro	Ala	195	200	205	
Leu	Ala	Tyr	Gly	Tyr	Ala	Gln	Asp	Gln	Glu	Pro	Asp	Ala	Arg	Gly	Arg	210	215	220	
Ile	Arg	Asn	Gly	Ala	Leu	Leu	Arg	Val	Tyr	Val	Pro	Arg	Ser	Ser	Leu	225	230	235	240
Pro	Gly	Phe	Tyr	Arg	Thr	Ser	Leu	Thr	Leu	Ala	Ala	Pro	Glu	Ala	Ala	245	250	255	
Gly	Glu	Val	Glu	Arg	Leu	Ile	Gly	His	Pro	Leu	Pro	Leu	Arg	Leu	Asp	260	265	270	

Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu
275 280 285

Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro
290 295 300

Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro
305 310 315 320

Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro
325 330 335

Gly Lys Pro Pro Arg Glu Asp Leu Lys
340 345

<210> 23
<211> 345
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Pseudomonas exotoxin A
cytotoxic fragment PE38 translocating and ADP ribosylating
domains with Arg at position 222 of PE38 (position 490 of
Pseudomonas exotoxin A) mutated to Ala

<400> 23
Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro
1 5 10 15

Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu
20 25 30

Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala
35 40 45

Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu
50 55 60

Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln
65 70 75 80

Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu
85 90 95

Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn
100 105 110

Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr
115 120 125

Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg
130 135 140

Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu Gln Ala His Arg Gln
145 150 155 160

Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu
165 170 175

Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln
 180 185 190
 Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala
 195 200 205
 Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro Asp Ala Ala Gly Arg
 210 215 220
 Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu
 225 230 235 240
 Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala
 245 250 255
 Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp
 260 265 270
 Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu
 275 280 285
 Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro
 290 295 300
 Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro
 305 310 315 320
 Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro
 325 330 335
 Gly Lys Pro Pro Arg Glu Asp Leu Lys
 340 345

<210> 24
 <211> 613
 <212> PRT
 <213> Pseudomonas sp.

<220>
 <223> Pseudomonas exotoxin A

<400> 24
 Ala Glu Glu Ala Phe Asp Leu Trp Asn Glu Cys Ala Lys Ala Cys Val
 1 5 10 15
 Leu Asp Leu Lys Asp Gly Val Arg Ser Ser Arg Met Ser Val Asp Pro
 20 25 30
 Ala Ile Ala Asp Thr Asn Gly Gln Gly Val Leu His Tyr Ser Met Val
 35 40 45
 Leu Glu Gly Gly Asn Asp Ala Leu Lys Leu Ala Ile Asp Asn Ala Leu
 50 55 60
 Ser Ile Thr Ser Asp Gly Leu Thr Ile Arg Leu Glu Gly Gly Val Glu
 65 70 75 80
 Pro Asn Lys Pro Val Arg Tyr Ser Tyr Thr Arg Gln Ala Arg Gly Ser
 85 90 95

Trp Ser Leu Asn Trp Leu Val Pro Ile Gly His Glu Lys Pro Ser Asn
 100 105 110
 Ile Lys Val Phe Ile His Glu Leu Asn Ala Gly Asn Gln Leu Ser His
 115 120 125
 Met Ser Pro Ile Tyr Thr Ile Glu Met Gly Asp Glu Leu Leu Ala Lys
 130 135 140
 Leu Ala Arg Asp Ala Thr Phe Phe Val Arg Ala His Glu Ser Asn Glu
 145 150 155 160
 Met Gln Pro Thr Leu Ala Ile Ser His Ala Gly Val Ser Val Val Met
 165 170 175
 Ala Gln Thr Gln Pro Arg Arg Glu Lys Arg Trp Ser Glu Trp Ala Ser
 180 185 190
 Gly Lys Val Leu Cys Leu Leu Asp Pro Leu Asp Gly Val Tyr Asn Tyr
 195 200 205
 Leu Ala Gln Gln Arg Cys Asn Leu Asp Asp Thr Trp Glu Gly Lys Ile
 210 215 220
 Tyr Arg Val Leu Ala Gly Asn Pro Ala Lys His Asp Leu Asp Ile Lys
 225 230 235 240
 Pro Thr Val Ile Ser His Arg Leu His Phe Pro Glu Gly Gly Ser Leu
 245 250 255
 Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro Leu Glu Thr Phe
 260 265 270
 Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu Glu Gln Cys Gly
 275 280 285
 Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala Ala Arg Leu Ser
 290 295 300
 Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu Ala Ser Pro Gly
 305 310 315 320
 Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln Pro Glu Gln Ala
 325 330 335
 Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu Arg Phe Val Arg
 340 345 350
 Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn Ala Asp Val Val
 355 360 365
 Ser Leu Thr Cys Pro Val Ala Ala Gly Glu Cys Ala Gly Pro Ala Asp
 370 375 380
 Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr Gly Ala Glu Phe
 385 390 395 400
 Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg Gly Thr Gln Asn
 405 410 415

Trp Thr Val Glu Arg Leu Leu Gln Ala His Arg Gln Leu Glu Glu Arg
 420 425 430
 Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu Glu Ala Ala Gln
 435 440 445
 Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln Asp Leu Asp Ala
 450 455 460
 Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala Leu Ala Tyr Gly
 465 470 475 480
 Tyr Ala Gln Asp Gln Glu Pro Asp Ala Arg Gly Arg Ile Arg Asn Gly
 485 490 495
 Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu Pro Gly Phe Tyr
 500 505 510
 Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala Gly Glu Val Glu
 515 520 525
 Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp Ala Ile Thr Gly
 530 535 540
 Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu Gly Trp Pro Leu
 545 550 555 560
 Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro Thr Asp Pro Arg
 565 570 575
 Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro Asp Lys Glu Gln
 580 585 590
 Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro Gly Lys Pro Pro
 595 600 605
 Arg Glu Asp Leu Lys
 610

<210> 25
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:two-step
 overlap PCR upstream mutagenic primer A

<400> 25
 gaacccgacg cagccggccg tatccgcaac

30

<210> 26
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:two-step
 overlap PCR downstream mutagenic primer B

<400> 26
 gttgcggata cggccggetg cgtcgggttc 30

<210> 27
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:two-step
 overlap PCR mutagenic primer C

<400> 27
 gctgtcgtgg aaccaggtcg accagg 26

<210> 28
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:two-step
 overlap PCR mutagenic primer D

<400> 28
 ctttgtagc agccgaattc atattcgat 29

<210> 29
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:RFB4 VH chain
 CDR3 in which "SSY" mutated to "THW"

<400> 29
 Gly Thr His Trp
 1

<210> 30
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:carboxyl
 terminal native sequence binding KDEL recycling
 receptor for transport of construct into cytosol
 from endoplasmic reticulum

<400> 30
 Arg Glu Asp Leu Lys
 1 5